Developmental Cell, Vol. 8, 803–816, June, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.05.007

Polarity Proteins in Axon Specification and Synaptogenesis

Review

Giselle R. Wiggin,[1,3] James P. Fawcett,[1,3] and Tony Pawson[1,2,*]
[1]Samuel Lunenfeld Research Institute
Mount Sinai Hospital
600 University Avenue
Toronto, Ontario M5G 1X5
Canada
[2]Department of Medical Genetics and Microbiology
University of Toronto
1 Kings College Circle
Toronto, Ontario M5S 1A8
Canada

The neuron is a prime example of a highly polarized cell. It is becoming clear that conserved protein complexes, which have been shown to regulate polarity in such diverse systems as the *C. elegans* zygote and mammalian epithelia, are also required for neuronal polarization. This review considers the role of these polarity proteins in axon specification and synaptogenesis.

Introduction

The ability of cells to polarize is critical for complex biological activities, such as the organization of the nervous system. Indeed, neurons are among the best examples of a highly differentiated and polarized cell type, typically extending a long thin axon, which is engineered to propagate signals, and several shorter and thicker dendrites, which are designed to receive signal inputs. The transfer of information from a neuron to its target occurs at the synapse, which are composed of specialized pre- and postsynaptic structures. The presynaptic terminal stores vesicles, which upon activation release neurotransmitters into the synaptic space, where they act on postsynaptic receptors. The asymmetric localization of proteins within the axon, dendrite, and synapse is essential for a neuron to establish its functional architecture.

A wealth of recent data has identified a number of gene products that have the capacity to impose cellular asymmetry, in part through their ability to form dynamic multiprotein complexes. Here we will consider the notion that such polarity proteins participate in axon specification, growth, and synaptogenesis, in part through signaling to the actin and microtubule cytoskeletons.

Polarity Complexes

The prototypic PAR (for partitioning-defective) genes were identified in *C. elegans* for their roles in directing asymmetric cell division during early development (Cowan and Hyman, 2004), and they encode proteins with catalytic and interaction domains characteristic of cellular signaling. PAR-1 and PAR-4 are serine/threonine kinases, as is the atypical protein kinase C (aPKC), which is also implicated in the regulation of polarity (Guo and Kemphues, 1995; Watts et al., 2000; Izumi et al., 1998). PAR-2 is a RING finger protein (Levitan et al., 1994), PAR-5 a 14-3-3 protein, which recognizes phosphorylated serine/threonine motifs (Morton et al., 2002), and both PAR-3 and PAR-6 are PDZ-domain-containing proteins (Etemad-Moghadam et al., 1995; Hung and Kemphues, 1999).

Experiments employing the mammalian and *Drosophila* orthologs of these *C. elegans* PAR proteins have shown that they can directly interact with one another to form larger multiprotein complexes. For example, PAR-6 associates with aPKC through its N-terminal PB1 domain, with GTP bound Cdc42 or Rac through a partial CRIB motif, and with PAR-3 through its PDZ domain, to form a subapical complex in epithelial cells (Hung and Kemphues, 1999; Joberty et al., 2000; Lin et al., 2000; Garrard et al., 2003).

In addition to PAR-3, PAR-6 and aPKC also associate with the tumor-suppressor protein Lethal giant larvae (Lgl, a large protein containing 14 WD40 repeats), leading to Lgl phosphorylation and functional inactivation (Plant et al., 2003; Betschinger et al., 2003; Yamanaka et al., 2003). PAR-6 can also bind the MAGUK protein Pals1, which is apically localized in epithelial cells (Hurd et al., 2003a; Wang et al., 2004).

These dynamic PAR-6/aPKC complexes therefore provide a biochemical link between distinct sets of polarity proteins that localize along an apical-basal axis in epithelial cells. These include the apical Crumbs/Pals/Patj complex, the PAR-3/PAR-6/aPKC complex, and the basolateral Scribble/Lgl/Dlg complex (Bilder, 2004; Macara, 2004). Consistent with the biochemical data, genetic analyses in *Drosophila* indicate that the PAR-3, Crumbs, and Scribble complexes have mutually interdependent functions (Tanentzapf and Tepass, 2003; Bilder et al., 2003). For example, the apical localization of the Crumbs/Pals/Patj complex is dependent on the PAR-3/PAR-6/aPKC polarity cassette (Nam and Choi, 2003; Hurd et al., 2003a; Tanentzapf and Tepass, 2003). The Crumbs complex in turn antagonizes the Scribble/Lgl/Dlg complex by regulating the size of the apical domain and maintaining the position of the PAR-3/PAR-6/aPKC complex (Tanentzapf and Tepass, 2003; Bilder et al., 2003). Together these polarity complexes define specific apical-basolateral domains of epithelial cells and the formation of specialized cell junctions. It is therefore pertinent to consider whether similar forces are at work in establishing polarity and specialization in neuronal cells.

Axon Specification

The initial event in establishing a polarized neuron is the specification of a single axon. How does one, and only one, neurite commit to become an axon, and how do the others remain as dendrites? Both the establishment and maintenance of neuronal polarity involve coordinated and widespread regulation of the cytoskeleton and membrane-trafficking machinery.

*Correspondence: pawson@mshri.on.ca
[3]These authors contributed equally to this work.

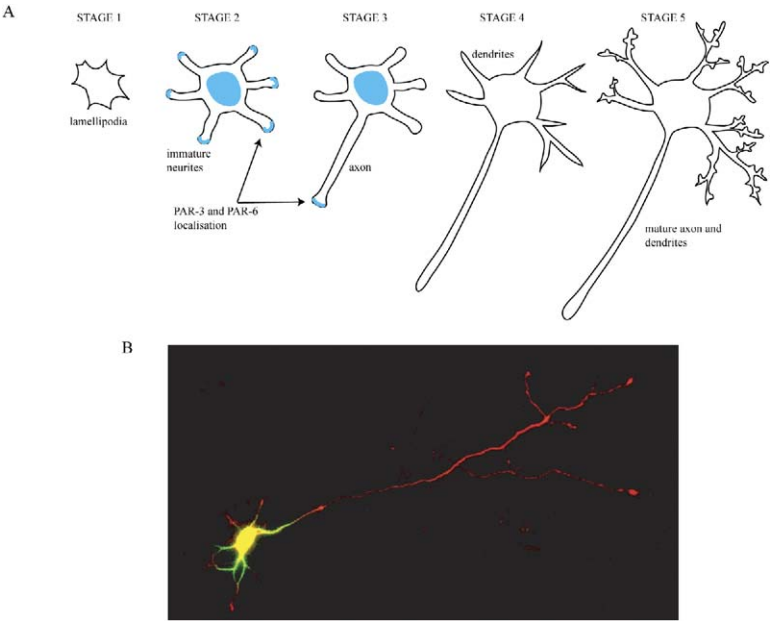

Figure 1. Localization of PAR-3 and PAR-6 in Axon Development

(A) Axon development and localization of PAR-3 and PAR-6.

(B) PAR-3 staining (red) in a stage 3 hippocampal neuron showing localization of PAR-3 to the axon and tips of developing growth cones. MAP-2 staining (yellow/green) is localized to the cell body and dendrites.

The establishment of neuronal polarity has been studied extensively through the culturing of pyramidal neurons from the rodent hippocampus. This in vitro differentiation process has been divided into five stages (Figure 1A; Dotti et al., 1988). Shortly after plating, neurons form lamellipodia (stage one). Neurons then extend several short processes called neurites, which grow to around 20 μm before undergoing a period of extension and retraction (stage 2). Within 24 hr, one of the neurites (the future axon) begins to elongate very rapidly, whereas the others (the future dendrites) undergo little extension (stage 3). After several days, the remaining neurites begin to grow and acquire the characteristics of dendrites (stage 4). The axon and dendrites then reach maturation, neurons form synaptic contacts, and spontaneous electrical activity propagates throughout the neural network (stage 5). This in vitro system by no means recapitulates all aspects of neuronal polarization in vivo, where extrinsic signals from the surrounding cellular environment likely play a major role in axon and dendrite development. However, the culturing of hippocampal neurons is a convenient way to examine some of the intrinsic mechanisms that govern axon specification.

The neurite destined to become an axon shows a number of particular characteristics prior to formation of the axon itself. For example, the axon-to-be develops a larger growth cone and accumulates a larger number of organelles and a higher concentration of cytosolic proteins and ribosomes (Fukata et al., 2002a; Horton and Ehlers, 2003). Since the motility of neurite growth cones is a consequence of high actin turnover, it follows that increased actin instability may also be a trigger for axonal specification (Bradke and Dotti, 1999). Indeed, global application of actin-depolymerizing drugs results in the development of neurons with multiple axons, whereas localized cytochalasin D treatment to an individual neurite causes this projection alone to become an axon. An unstable, loose actin meshwork may therefore allow microtubules to protrude into distal areas of the growth cone, thereby promoting axon elongation (Bradke and Dotti, 1999). Proteins associated with microtubules also influence axon specification, including collapsin response mediator protein-2 (CRMP-2) and tau, which are preferentially associated with axonal microtubules.

Therefore, the regulation of both the actin and microtubule networks appears to be a key determinant in axon development. The Rho family of GTPases play an important role in controlling the actin cytoskeleton and microtubule orientation, and the phosphorylation of microtubule-associated proteins regulates microtubule stability and polymerization. Recent evidence places the PAR-3/PAR-6 complex as a potential hub in both of these processes.

PAR Proteins and the Rho Family of GTPases in the Axon

Before the specification of a single axon (stage 2), PAR-3 is localized in the cell body and at the tips of all nascent processes (Shi et al., 2003; Schwamborn and Puschel, 2004; Nishimura et al., 2005). By stage 3, PAR-3 is lost from dendrites and becomes selectively expressed in the axon and developing growth cone (Figure 1B; Shi et al., 2003). The spatial and temporal expression of PAR-6 during axon specification is similar to PAR-3, being confined to the cell body and the axon by stage 3 of development (Shi et al., 2003; Schwamborn and Puschel, 2004). By contrast, when overexpressed in neurons, PAR-3 and PAR-6 fail to localize correctly, leading to defects in polarization (Shi et al., 2003; Schwamborn and Puschel, 2004; Nishimura et al., 2005). Most of these neurons fail to elaborate a single axon, but instead contain two or more neurites of a similar length, which show abnormal microtubule organiza-

tion and do not express markers of mature axons (Shi et al., 2003; Nishimura et al., 2005).

The Rho family members Rac1 and Cdc42 regulate distinct facets of the actin cytoskeleton and both can bind to the PAR-3/PAR-6 complex (Joberty et al., 2000; Lin et al., 2000; Qiu et al., 2000). Consistent with a role for Cdc42 in axon specification, a hyperactive Cdc42 mutant that autonomously cycles between GDP and GTP bound forms (Cdc42L28) induces the formation of supernumerary axons (Schwamborn and Puschel, 2004). By contrast, neurons transfected with the constitutively active Cdc42V12 mutant show a reduction in neurite number (Nishimura et al., 2005) or fail to extend any neurites (Schwamborn and Puschel, 2004). Similarly, the inactive Cdc42N17 mutant also decreased the number of cells expressing a single axon-like neurite (Nishimura et al., 2005). This suggests that the cycling of Cdc42 between the GDP and GTP bound forms is essential for its function in neuronal polarity, as is the case for cell polarity in yeast (Irazoqui et al., 2003). Furthermore, RNAi-induced knockdown of Cdc42 leads to a complete loss of polarity, such that the majority of neurons fail to form axons, although the number and size of minor neurites was unaffected (Schwamborn and Puschel, 2004).

Do Cdc42 and the PAR-3/PAR-6 complex colocalize during axonal specification? This issue remains somewhat ambiguous, since different studies have yielded distinct patterns of neuronal Cdc42 localization (Santos Da Silva et al., 2004; Schwamborn and Puschel, 2004). Nonetheless, Cdc42 does appear to overlap, at least to some extent, with PAR-3 and PAR-6 during neuronal polarization. Rac1 can also bind PAR-6, albeit more weakly than Cdc42 (Johansson et al., 2000; Lin et al., 2000), and as with Cdc42, both constitutively active and dominant-negative Rac1 impaired neurite outgrowth and axon specification (Nishimura et al., 2005). In addition, the Rap1B GTPase has been suggested to act upstream of Cdc42 in axonal specification (Schwamborn and Puschel, 2004).

Interestingly, recent data suggest that the PAR-3/PAR-6/aPKC complex may not only be a downstream target of Rho family GTPases, but may directly influence Rac activation by regulating the guanine nucleotide exchange factors (GEFs) that convert Rac from the inactive GDP bound state to an active GTP bound state. Two recent papers have shown that closely related Rac-specific GEFs, Tiam1 and STEF (also known as Tiam2), bind directly to PAR-3 (Nishimura et al., 2005; Chen and Macara, 2005; reviewed in Hurd and Margolis, 2005), and one report indicates that this interaction influences axonal specification (Nishimura et al., 2005). Previous work has shown that overexpression of Tiam1 leads to the development of multiple axon-like neurites in cultured hippocampal neurons, implicating this GEF in axonal formation (Kunda et al., 2001). Nishimura et al. (2005) found that STEF directly interacts with the C-terminal region of PAR-3 and accumulates at the axon tip in stage 3 neurons, where it colocalizes with PAR-3. As with Tiam1, overexpression of STEF promoted neurite growth and generated multiple axon-like neurites, a phenotype recapitulated with the fragment of PAR-3 that binds STEF (Nishimura et al., 2005). However, these axon-like neurites did not have all the features of a mature axon, suggesting that additional components are necessary for full maturation. By contrast, the expression of a fragment of STEF encoding just the central portion of the protein inhibits neurite growth, with most cells arresting at the stage 2-3 transition. This fragment of STEF, although able to interact with PAR-3, does not contain the GEF domain, so it is unable to activate Rac and thus may act as a dominant negative. These results suggest a model in which Rac activation, through STEF, is necessary for axonal specification and that PAR-3 functions as a regulatory molecule for this process.

The role of PAR-3 in activating STEF and hence Rac could be neuron specific since, in epithelial cells, PAR-3 appears to inhibit Tiam1 and Rac to stimulate tight junction assembly (Chen and Macara, 2005). Although it is possible that STEF and Tiam1 are differentially regulated by PAR-3, both Tiam1 and STEF yield multiple axon-like neurites in cultured hippocampal neurons when overexpressed (Kunda et al., 2001; Nishimura et al., 2005; Hurd and Margolis, 2005). While there are important mechanistic details to be uncovered, these results are significant in linking the PAR-3 polarity protein to a specific Rac GEF, and thus to a role in axon specification. A more detailed discussion of the role of Rho GTPases in neuronal polarity can be found in Govek et al. (2005).

Potential Mechanisms for Localizing Polarity Proteins in Neurons

The correct localization of PAR-3 and PAR-6 to the tip of a developing axon appears to play a role in axon specification. So, how is this complex, and other multiprotein assemblies involved in polarity, localized in neurons to promote axon development?

Phosphatidylinositol 3′ Kinase

One mechanism for directing the PAR complex to the axon tip may be via the localized activation of phosphatidylinositol 3′ kinase (PI3K). Activated PI3K converts $PI(4,5)P_2$ into $PI(3,4,5)P_3$. This phospholipid recruits PH domain-containing proteins to the plasma membrane, such as PDK1 (which can activate aPKC) and several GEFs (e.g., Vav, P-Rex1) that in turn stimulate the activity of the Rho family of GTPases such as Cdc42 and Rac1 (Chou et al., 1998; Le Good et al., 1998; Han et al., 1998; Welch et al., 2002). Both aPKC and Cdc42/Rac1 interact with PAR-3 and PAR-6 and could therefore recruit them to localized regions of PI3K activity (Shi et al., 2003; Menager et al., 2004). Since Rho family GTPases have the potential to activate PI3K (Weiner et al., 2002; Wang et al., 2002), the recruitment of GEFs could set up a positive feedback loop, eliciting a burst of PIP_3 signaling at the axon tip, strengthening the growth response locally to bring about axon development.

Indeed, active PI3K (detected by a GFP-tagged form of the Akt/PKB PH domain, or via the phosphorylation of Akt/PKB itself) is enriched selectively at the tip of axons (Shi et al., 2003; Menager et al., 2004). A recent publication has demonstrated that localized TrkA activity, stimulated by the plasma membrane ganglioside sialidase, can lead to the activation of PI3K at the tip of a single neurite (Santos Da Silva et al., 2005; reviewed

by Jiang and Rao, 2005). PI3K inhibitors, such as LY 294002, delay the transition from stage 1 to stage 3 neurons, affecting both axon formation and elongation (Shi et al., 2003; Menager et al., 2004). When an immature neurite contacts a bead coated in laminin, a substrate known to induce axon specification, PIP_3 accumulates in the growth cone, and this is followed by rapid neurite elongation (Menager et al., 2004).

Overexpression of PTEN, a lipid phosphatase that counteracts the actions of PI3K, prevents PAR-3 localization to neurites and inhibits axonal formation (Shi et al., 2003; Jiang et al., 2005). Conversely, downregulating PTEN expression by siRNA increased the number of axons, while reducing the number of dendrites (Jiang et al., 2005). Interestingly, PAR-3 has recently been shown to directly interact with PTEN in *Drosophila* epithelia and neuroblasts (von Stein et al., 2005). Thus, one can envision an inhibitory feedback loop, whereby PI3K recruits PAR-3 to the axon tip, which in turn recruits PTEN to inhibit PI3K activity. This could either control burgeoning PIP_3 levels at the axon tip or suppress PIP_3 at surrounding sites to establish a sharp gradient of PIP_3 signaling. Furthermore, the PIP_2 generated by PTEN could be involved in regulating the actin cytoskeleton by binding directly to actin-associated proteins or to proteins such as WASP that stimulate actin polymerization (von Stein et al., 2005).

PAR Proteins and LIMK, a Regulator of the Actin Cytoskeleton

LIM kinase 1 (LIMK1) is directly activated by PAK1, which is itself a downstream target of Rac1 and Cdc42 (Edwards et al., 1999). Overexpression of LIMK1 initially accelerates axon formation and enhances the accumulation of phosphorylated Akt/PKB, PAR-3, and PAR-6 at growth cones (Rosso et al., 2004). While the overexpression of PAR-3 alone led to defective neuronal polarization (Shi et al., 2003), the coexpression of PAR-3 and LIMK1 induced the extension of a single, highly branched axon, with PAR-3 accumulated at growth cones and branch points, suggesting that LIMK1 is required for the localization of PAR-3 to a single neurite (Rosso et al., 2004). In addition, neurons overexpressing a nonphosphorylatable mutant of the LIMK1 substrate cofilin failed to localize PAR-3 (Rosso et al., 2004), suggesting that the cofilin-dependent regulation of the actin cytoskeleton is required for correct localization of PAR-3. In vivo, the activation of LIMK1 is likely to be tightly regulated since its prolonged overexpression induces axon retraction and growth-cone collapse (Rosso et al., 2004).

Role of KIF3A and APC in PAR-3 Localization

A potential mechanism for PAR-3 transport to the distal tip of the axon is through interactions with the plus end-directed microtubule motor protein KIF3A (also known as kinesin II) and adenomatous polyposis coli (APC) (Nishimura et al., 2004a; Shi et al., 2004). The KIFs are a large family of molecular motors that function as the main long-distance transporters toward the periphery of neurons (Setou et al., 2004). APC also binds the plus end of microtubules and stimulates microtubule assembly and bundling (Bienz, 2002).

Two predicted coiled-coil domains in the C terminus of PAR-3 can bind to the tail region of KIF3A (Nishimura et al., 2004a). The expression of dominant-negative PAR-3 and KIF3A fragments that disrupt PAR-3 binding to KIF3A inhibit the accumulation of PAR-3 and aPKC at the tip of neurites, abolishing neuronal polarity (Nishimura et al., 2004a). Significantly, PAR-3, PAR-6, and aPKC also colocalize with KIF3A in MDCK cells, and it is suggested that by binding to motor proteins, the PAR complex could regulate the polarized trafficking of proteins along microtubules (Fan et al., 2004). In addition, PKCλ activity is required in 3T3-L1 adipocytes for the loading of KIF3A onto microtubules and the subsequent exocytosis of GLUT4-containing vesicles, suggesting that KIF3A may be a PKCλ substrate (Imamura et al., 2003).

In nonpolarized cells, APC is found in the cell body and at the tips of undifferentiated neurites, but becomes selectively enriched at the tip of the tau-positive nascent axon following polarization, partially colocalizing with PAR-3. The overexpression of full-length or truncated mutants of APC prevented PAR-3 from localizing correctly and abolished neuronal polarity. APC could also be found with PAR-3 in puncta along the shaft of the nascent axon, suggesting that APC may be involved in transporting PAR-3 to the tips of developing axons (Shi et al., 2004). In epithelial cells, APC can also bind to Dlg, which genetically interacts with Scribble and Lgl to influence the distribution of the PAR-3/PAR-6 complex (Matsumine et al., 1996).

Growing data, therefore, suggest that proteins known to regulate polarity in epithelial cells also have a role in neuronal polarity. The localization of PAR-3, PAR-6, and their interactors to the axon tip in hippocampal neurons appears important in specifying which neurite is to become an axon, and in the process of axon outgrowth. However, a recent observation has illustrated the necessity for further studies on the role of the PAR-3/PAR-6 complex in neurons. *Drosophila* null mutants of Baz (*Drosophila* PAR-3), PAR-6, and aPKC show normal axon specification and outgrowth in mushroom body neurons (Rolls and Doe, 2004). In contrast to the observations made in mouse hippocampal neurons, Baz and PAR-6 were not localized to axonal tips in *Drosophila*, nor did the overexpression of Baz or PAR-6 have any effect on neuronal polarity in aCC or mushroom body neurons.

One possible reason for this difference is that mammalian neurons require PAR-complex proteins for axonal specification, whereas invertebrate neurons do not. There is some precedence for such differences. For example, the expression of constitutively active Rac in *Drosophila* promotes neurite extension (Luo et al., 1994), whereas in mouse hippocampal neurons the same Rac1 mutant impairs neurite growth and axon specification (Nishimura et al., 2005). Similarly, in mammalian epithelial cells, PAR-3 has no effect on adherens junction function (Chen and Macara, 2005), whereas in *Drosophila* adherens junctions, Baz is required (Harris and Peifer, 2004).

Alternatively, mammalian PAR-3 and PAR-6 may be less important for specifying axon identity in vivo than is suggested by their role in hippocampal neurons in vitro, cultured in the absence of external polarity cues. In vivo, the process of axon specification will be strongly influenced by contact with surrounding cells and by signals that these cells generate. In order to

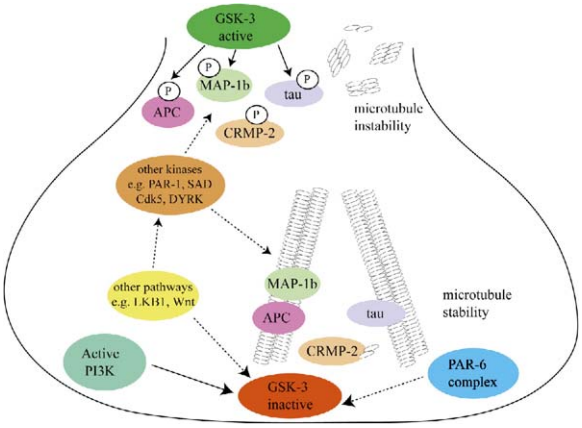

Figure 2. Signaling to Microtubule-Associated Proteins in the Developing Growth Cone

further elucidate the role of the PAR proteins in mammalian neuronal polarization, it will be necessary to examine this process in vivo, or in brain slices, where the native cellular environment is maintained. Neuron-specific knockouts of the PAR proteins and their interactors in mice should prove to be informative in this respect. In addition, the use of real-time imaging would allow the dynamics of this system to be visualized.

Protein Kinases, Microtubules, and Neuronal Polarity

Signaling pathways can directly regulate the microtubule dynamics required for neuronal polarity by regulating the phosphorylation of microtubule-associated proteins. Here we focus on the role of GSK-3 and the PAR-1 family of kinases in this process.

GSK-3 Signaling and Neuronal Polarity

Phosphorylation of APC by GSK-3 prevents the interaction between APC and microtubules (Zumbrunn et al., 2001). At stage 2 of hippocampal neuron differentiation, GSK-3β protein is uniformly distributed throughout the neuron; however, the inactive form of GSK-3β (which is phosphorylated at Ser-9) is concentrated at the tip of each neurite (Shi et al., 2004; Jiang et al., 2005). By stage 3, phospho-GSK-3β is enriched at the tip of the axon, rather than the dendrites (Shi et al., 2004). Therefore, at the tip of axons, where GSK-3 is inactive, APC would remain unphosphorylated and therefore able to interact with and stabilize the growing ends of microtubules (Figure 2; Zumbrunn et al., 2001; Zhou et al., 2004). This was also observed in dorsal root ganglion (DRG) neurons, where a pool of phosphorylated, inactive GSK-3 is localized to the growth cone, colocalizing with both APC and F-actin (Eickholt et al., 2002; Zhou et al., 2004).

Regulation of GSK-3 Activity

So, how is GSK-3 inactivated specifically at the tip of axons? GSK-3 is an unusual kinase, being active in unstimulated cells and inactivated downstream of a number of signaling pathways, including Wnt, MAPK, and PI3K (Jope and Johnson, 2004; Patel et al., 2004). Inhibition of GSK-3 by the PI3K and MAPK pathways occurs via the phosphorylation of a key N-terminal residue (Ser-21 in GSK-3α and Ser-9 in GSK-3β). The mechanism by which the Wnt pathway inhibits GSK-3 is not fully understood but is believed to be distinct from that used by the PI3K and MAPK pathways (Jope and Johnson, 2004; Patel et al., 2004).

APC and GSK-3 exist together in a multiprotein complex, including axin and β-catenin, that is regulated by Wnt signaling and thought to be inaccessible to the PI3K pathway (Bienz, 1999; Frame and Cohen, 2001). However, recent evidence suggests that PI3K activity may be able to regulate components of this complex in neurons (Zhou et al., 2004). As described above, PI3K and Akt/PKB are activated specifically at axon tips, which coincides with the localization of inhibited GSK-3. Akt/PKB directly phosphorylates and inactivates GSK-3 and thus would seem a likely candidate in mediating GSK-3 inactivation at the axon tip. Indeed, treatment of neurons with the PI3K inhibitor, LY 294002, at least partially blocked the phosphorylation of GSK-3β at Ser-9 (Jiang et al., 2005). Similarly, the BDNF- or NT3-induced phosphorylation of GSK-3β could be prevented by another PI3K inhibitor, wortmannin (Yoshimura et al., 2005).

A PAR-6 Complex in GSK-3 Regulation

PAR-6 and aPKC have been shown to regulate migration in astrocytes and cerebellar granule neurons by controlling centrosome positioning (Etienne-Manneville and Hall, 2003; Solecki et al., 2004). Interestingly, GSK-3 has been placed downstream of the PAR-6/PKCζ/Cdc42 complex in the regulation of astrocyte migration (Etienne-Manneville and Hall, 2003; reviewed by Harwood and Braga, 2003). In these cells, the inactivation of GSK-3β is independent of PI3K but dependent on Cdc42 and PKCζ activity. PAR-6 and PKCζ interact with GSK-3β to position the centrosome and control the direction of cell protrusion. Phosphorylation (i.e., inactivation) of GSK-3β occurs specifically at the leading edge of migrating astrocytes. This causes GSK-3β to dissociate from PKCζ and induces the interaction of APC with microtubules, which promotes cell polarization (Etienne-Manneville and Hall, 2003).

Therefore, this process appears to be similar to the regulation of APC at axonal tips. Indeed, hippocampal neurons treated with a specific peptide inhibitor of PKCζ showed defects in APC localization (Shimomura et al., 2005). However, there is no direct biochemical evidence that PKCζ inhibits GSK-3. Although PKCζ has been shown to (weakly) phosphorylate GSK-3β in vitro, this phosphorylation did not occur at Ser-9 and was not sufficient to inhibit GSK-3 activity toward a peptide substrate (Oriente et al., 2001). In addition, the treatment of neurons with the PKC inhibitor, bisindolylmalemide I, did not prevent the phosphorylation of GSK-3β at Ser-9 (Jiang et al., 2005). However, caution must be taken when interpreting data obtained with bisindolylmalemide I since, in addition to PKC, it also inhibits GSK-3 and a number of other protein kinases (Hers et al., 1999; Davies et al., 2000).

LKB1/PAR-4 and PAR-1 in GSK-3 Regulation

The LKB1/PAR-4 polarity kinase has been shown to enhance Wnt signaling and regulate GSK-3 activity. In *Xenopus*, LKB1, GSK-3, and PKCζ can be coimmunoprecipitated, suggesting that they form a complex

(Ossipova et al., 2003; reviewed by Clements and Kimelman, 2003). A reduction in LKB1 levels led to a decrease in GSK-3β phosphorylation at Ser-9, rendering GSK-3 more active. This suggests that LKB1 promotes the inhibition of GSK-3, perhaps via PKCζ (Ossipova et al., 2003). However, two other studies have implicated LKB1 in the inhibition of the Wnt pathway and subsequent activation of GSK-3 (Spicer et al., 2003; Lin-Marq et al., 2005), so further investigation is required to determine the role of LKB1 in this pathway. Another polarity regulator, PAR-1 (also known as MARK), has been reported to be a Dishevelled-associated kinase and a positive regulator of the Wnt signaling pathway, resulting in the inhibition of GSK-3 (Sun et al., 2001).

Interestingly, PAR-1 itself can be activated by LKB1 (Spicer et al., 2003; Lizcano et al., 2004), as well as by Tao1/MARKK, a Ste20 family kinase (Timm et al., 2003). Significantly, there is considerable interaction between LKB1, PAR-1, and the PAR-3/PAR-6/aPKC complex. As mentioned above, aPKC can interact with LKB1 (Ossipova et al., 2003). aPKC phosphorylates PAR-1, leading to the binding of 14-3-3 proteins and the relocalization of PAR-1 from the membrane to the cytoplasm (Kusakabe and Nishida, 2004; Suzuki et al., 2004; Hurov et al., 2004). This phosphorylation of PAR-1 by aPKC may also inhibit PAR-1 kinase activity (Hurov et al., 2004). PAR-1 in turn phosphorylates PAR-3, creating binding sites for the 14-3-3 protein PAR-5 (Benton and St Johnston, 2003; Hurd et al., 2003b) and thus altering PAR-3 localization. As noted below, two kinases closely related to PAR-1 (SAD-A/B) appear to be important for axonal growth in vivo (Kishi et al., 2005) and are potentially regulated via these interacting pathways (Figure 3).

Effects of GSK-3 Inhibitors on Neuronal Polarity

As inhibited GSK-3 appears to be confined to a discrete region of the growing axon, what would be the effect of applying pharmacological inhibitors of GSK-3 globally to neurons growing in culture? Two recent papers report that the inhibition of GSK-3 by a variety of pharmacological agents, or an shRNA-induced reduction in GSK-3 expression, significantly increased the number of neurons with multiple axons (which tended to be shorter than usual), with a consequent decrease in the number of dendrites (Jiang et al., 2005; Yoshimura et al., 2005). Time-lapse microscopy showed that the GSK-3 inhibitor, SB 415286, reduced the time neurites spent retracting but increased the time spent on growth, leading to the net effect of promoting neurite elongation (Jiang et al., 2005). Significantly, constitutively active, myristoylated Akt/PKB (which would be expected to constitutively inhibit GSK-3) also led to the formation of multiple axons (Jiang et al., 2005).

Multiple axons were also observed even if the GSK-3 inhibitors were not applied until stage 3 of neuron development, by which time one neurite was already "selected" to become an axon (Jiang et al., 2005). This indicates that inhibiting GSK-3 can influence both the formation and maintenance of neuronal polarity. It also demonstrates that GSK-3 inhibition can convert a preexisting dendrite into an axon. This leads to the suggestion that axon formation is the default state, which has to be actively and continuously suppressed by GSK-3. Indeed, several mechanisms have been reported to transform dendrites into axons, but none so far has been shown to convert axons into dendrites (Jiang et al., 2005).

GSK-3 Regulation of CRMP

APC is not the only microtubule binding protein regulated by the localized activation of GSK-3 in neurons. CRMP-2 binds to tubulin dimers and promotes their assembly into microtubule polymers (Fukata et al., 2002b). At least four serine and threonine residues in CRMP-2 can become phosphorylated, and phosphorylated CRMP is less able to interact with tubulin. It has recently been shown that CRMP-2 can be phosphorylated by GSK-3, following the addition of a priming phosphate by DYRK or Cdk5 (Cole et al., 2004; Yoshimura et al., 2005).

Overexpression of CRMP-2 increases axonal length and branching (Fukata et al., 2002b; Yoshimura et al., 2005). It also induces the formation of multiple axons and can even cause aberrant axons to sprout from established dendrites (Inagaki et al., 2001; Fukata et al., 2002b; Cole et al., 2004; Yoshimura et al., 2005). This is the same phenotype observed following treatment of neurons with GSK-3 inhibitors. A truncated, dominant-negative mutant of CRMP-2 led to the formation of neurons with short or absent axons (Inagaki et al., 2001), and reducing CRMP-2 expression by siRNA caused a marked inhibition of NT-3- and BDNF-induced axon elongation and branching (Yoshimura et al., 2005).

The expression of GSK-3-insensitive CRMP-2 mutants also promoted axon outgrowth and branching in addition to stimulating the formation of supernumerary axons (Cole et al., 2004; Yoshimura et al., 2005). However Cole et al. (2004) show that a nonphosphorylatable mutant of CRMP-2 is less efficient at increasing axon length compared to wild-type CRMP-2, suggesting that GSK-3 phosphorylation of CRMP-2 stimulates axon growth. On the other hand, Yoshimura et al. (2005) find that a nonphosphorylatable CRMP-2 is actually more effective at stimulating axon growth than wild-type CRMP-2, while a phospho-mimicking CRMP-2 mutant is less effective than wild-type protein. This suggests that GSK-3 phosphorylation of CRMP-2 has a negative effect on axon growth. The reason for these contrasting results is not clear, but could be because different phosphorylation sites were mutated in the two studies.

However, the situation in vivo is highly dynamic and it is likely that both phosphorylated and unphosphorylated CRMP-2 are required to generate the cycles of microtubule extension and retraction that bring about axon outgrowth. During stage 3 of axon development, CRMP-2 would remain unphosphorylated at the axon tip where GSK-3 is inhibited and thus be more able to interact with tubulin. In the remainder of the neuron, CRMP-2 would be phosphorylated by active GSK-3, which would therefore downregulate microtubule assembly. Nonphosphorylatable CRMP-2 mutants can rescue the lack-of-axon phenotype brought about by the expression of active GSK-3β (Yoshimura et al., 2005). This suggests that regulating CRMP-2 phosphorylation is one of the major functions for GSK-3 in determining neuronal polarity. Significantly, Numb has been identified as a CRMP binding partner in the central region of axonal growth cones in hippocampal neurons (Nishimura et al., 2003). Numb also interacts with α-adaptin (Santolini et al., 2000), one of the four

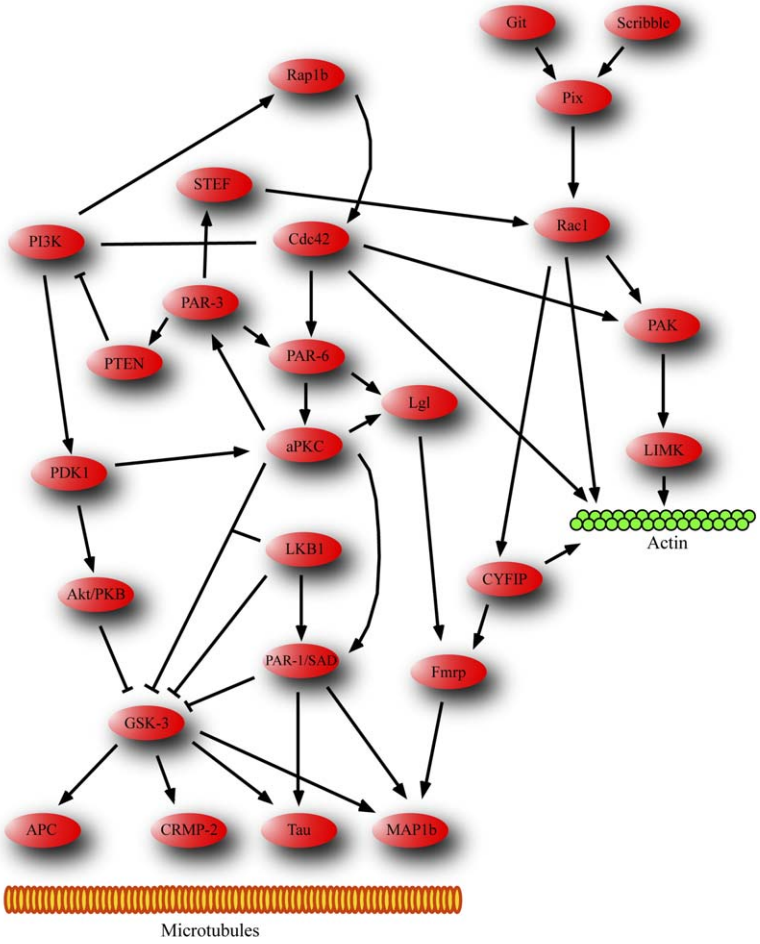

Figure 3. Signaling Pathways Regulating the Cytoskeleton Leading to Axon Specification and Synaptogenesis

subunits of the endocytic AP-2 complex, and it has been speculated that CRMP-2 may contribute to the establishment of neuronal polarity by regulating polarized Numb-mediated endocytosis at the axonal growth cone (Nishimura et al., 2003).

Regulation of Tau and MAP1b by Phosphorylation

Tau is a microtubule binding protein that has an established role in axon outgrowth and neuronal polarity (Mandell and Banker, 1996a). Tau phosphorylation by GSK-3 reduces tau binding to microtubules and thus impairs microtubule assembly (Stoothoff and Johnson, 2005). MAP1b can also be phosphorylated by GSK-3, and this results in the destabilization of microtubules (Goold et al., 1999; Trivedi et al., 2005). There is a spatial gradient of tau and MAP1b phosphorylation along the nascent axon, suggesting that the phosphorylation may be regulating neuronal polarity (Mandell and Banker, 1996b; Trivedi et al., 2005).

Although there is a correlation between GSK-3 phosphorylation of various microtubule binding proteins and neuron outgrowth, these proteins are in fact phosphorylated by several different protein kinases at multiple residues. It is more likely that the different combinations and permutations of phosphorylation events act together to regulate microtubule stability and neuronal polarity. Of significance, PAR-1 is also implicated in axonal outgrowth through its phosphorylation of tau and other MAPs on KXGS motifs. Mutating the KXGS sites to KXGA in tau almost abolished the outgrowth of neurites following application of a differentiation stimulus (Biernat et al., 2002). Transfection of wild-type (but not kinase-dead) MARK2 (PAR-1b) spontaneously triggers neurite outgrowth, whereas cotransfection with a KXGA mutant of tau prevented formation of these extended neurites. These data suggest that the elaboration of neurites is achieved by MARK2 phosphorylation of the KXGS motifs on tau. MARK2-induced phosphorylation causes tau to be released from microtubules and to partly associate with the actin network during neurite outgrowth (Biernat et al., 2002). PAR-1 has recently been shown to be the trigger for the temporally ordered phosphorylation of tau (Nishimura et al., 2004b; reviewed by Fortini, 2004), with phosphorylation of Ser-262 and Ser-356 by PAR-1 being a prerequisite for downstream kinases, including GSK-3 and Cdk5, to phosphorylate several additional sites.

Direct physiological evidence that a kinase regulates neuronal polarity through microtubules was recently provided by the analysis of SAD knockout mice (Kishi et al., 2005). Mammalian SAD-A and SAD-B (also known as BRSK1/2) are protein kinases with a catalytic domain most closely related to PAR-1. Expression of

these kinases is largely restricted to the nervous system and occurs early in the program of neuronal differentiation. In wild-type mice, SAD-A and SAD-B can be found in both axonal and dendritic compartments. Mice lacking both SAD isoforms show little spontaneous movement or response to tactile stimulation and die within 2 hr of birth. Neurons from these mice fail to form distinct axons or dendrites either in vivo or in culture. This observation indicates a cell-autonomous requirement for SAD-A and SAD-B in neuronal polarization (Kishi et al., 2005). This regulation of polarization may be accomplished through the phosphorylation of tau and other microtubule proteins. The overexpression of SAD-A increases tau phosphorylation at Ser-262, a site also targeted by PAR-1. Tau phosphorylated at Ser-262 is concentrated in the dendrites of wild-type cultured neurons, and there is a decrease in this tau phosphorylation in SAD-A/B-deficient mice (Kishi et al., 2005). This suggests that SAD kinases act in vivo to regulate polarity in neurons of the mammalian nervous system, through the phosphorylation of tau and the consequent alterations in microtubule organization. Since SAD isoforms are found in both axons and dendrites, their activity must be regulated differentially in these two compartments; upstream kinases such as LKB1 and Tao1 are likely candidates. It will be interesting to determine whether PAR-3 and PAR-6, which are known to interact with PAR-1, also play a role in regulating SAD-A and SAD-B. Mutants in the *C. elegans* ortholog, SAD-1, have defects in synaptogenesis (Crump et al., 2001). It will therefore be important to determine whether the SAD knockout mice are also unable to form proper synapses.

It can be seen, therefore, that the modulation of microtubule stability, via the phosphorylation of microtubule-associated proteins, is a major regulator of axon specification. Polarity proteins appear to have a role in this process. This can be a direct effect, in the case of the PAR-1 and SAD isoforms, or an indirect effect, via the modulation of GSK-3 activity, for example. Indeed, GSK-3 is proving to be a key player in neuronal polarity through its actions on cytoskeletal dynamics. Interestingly, just as SAD mutants have been shown to affect both axon specification and synaptogenesis (Crump et al., 2001), the *Drosophila* ortholog of GSK-3, Shaggy, also affects synaptic growth and development (Franco et al., 2004). We now go on to consider the role of other polarity regulators in synaptogenesis.

Synapse Development

Synapses are sites of cell-cell contact specialized for the transmission of chemical and electrical signals between neurons and their targets, activities that require the asymmetric distribution of proteins and mRNA within the pre- and postsynaptic compartments (Martin, 2004). Converging evidence from a number of studies suggests that polarity proteins modulate actin and microtubule dynamics at the synapse. Here we consider some pertinent examples of polarity regulators acting on the cytoskeleton to regulate synapse architecture and function.

At the presynaptic terminal, synaptic vesicles containing neurotransmitters translocate to an actin-rich region, called the active zone, where they dock and fuse with the plasma membrane (Dresbach et al., 2001; Ziv and Garner, 2004). The released neurotransmitters cross the synapse to the postsynaptic region, which is characterized by an electron-dense area, also highly enriched in actin, called the postsynaptic density (Ziff, 1997; Rao and Craig, 2000). This postsynaptic region contains many different scaffolding proteins, such as PSD-95, which binds directly to glutamate receptor family members and is important for localizing these receptors at or near the plasma membrane (Kim and Sheng, 2004). Much of our understanding of the role of polarity proteins in controlling synaptic structure and function comes from work involving the *Drosophila* larval neuromuscular junction (NMJ). The NMJ presynaptic terminal contains a large microtubule hairpin loop, and microtubule-associated proteins can control synaptic architecture and growth (Dresbach et al., 2001; Schaefer and Nonet, 2001). Thus, the regulation of actin and microtubule dynamics is required for the development and maintenance of the synapse.

Fragile X and Synapse Development

Synaptic plasticity can be regulated by local events within the synapse, including local transcription and translation, which occurs both during synaptic development and as a consequence of synaptic activity. Proper synapse formation is critical for the function of the nervous system, since disruption of the development or architecture of the synapse leads to profound neurological deficits, such as that seen in Fragile X syndrome, the most common form of inherited mental retardation (Chiurazzi et al., 2003). Fragile X syndrome is caused by the loss of Fragile X Mental Retardation protein (Fmrp), encoded by the FMR1 gene. Fmrp is enriched at the synapse and is thought to control transcriptional and translational events (Zhang and Broadie, 2005). Morphologically, patients with Fragile X syndrome have profound changes in synaptic architecture, including much longer dendritic spines and larger synaptic boutons. *Drosophila* Fmrp (dFmrp) is highly expressed both in the pre- and postsynaptic regions, and loss of expression leads to the abnormal development of dendritic spines and an increase in synaptic surface area (Zhang and Broadie, 2005), similar to the defects in the murine knockout (Kooy, 2003) and human disease (Chiurazzi et al., 2003).

dFmrp is an RNA binding protein and interacts directly with Futsch mRNA, which encodes an ortholog of mammalian MAP1b (Zhang et al., 2001). Functionally, Futsch expression is downregulated by the overexpression of dFMR1 (Zhang et al., 2001), and Futsch loss-of-function mutants suppress loss-of-function defects in dFMR1 mutants, suggesting that Fragile X protein negatively regulates Futsch expression (Zhang et al., 2001). dFmrp has been implicated in synapse formation in *Drosophila* by binding directly to cytoplasmic Fmrp-interacting protein (CYFIP/Sra), itself a Rac binding protein (Schenck et al., 2003). The Rho family of GTPases are known to be important regulators of synaptic development (Ramakers, 2002; Govek et al., 2005), and when GTP bound Rac binds CYFIP/Sra, it releases dFmrp from CYFIP/Sra, thereby allowing Fmrp to bind Futsch mRNA and downregulate Futsch expression in the syn-

apse, leading to marked changes in synaptic architecture (Schenck et al., 2003).

Intriguing new data link Lgl to this pathway, since Lgl has emerged as a suppressor of dFMR1 gain-of-function mutations (Zarnescu et al., 2005). In both *Drosophila* and mice, Lgl forms a stable complex with Fmrp, and in *Drosophila*, this complex can modulate the size of the neuromuscular junction (Zarnescu et al., 2005). As noted, Lgl associates with PAR-6 and aPKC, and indeed mouse Fmrp can be phosphorylated by aPKC in vitro (Zarnescu et al., 2005). Genetically, aPKC loss-of-function alleles suppress the synaptic hyperplasia observed in dFMR1 homozygote null flies, demonstrating that aPKC and dFMR1 function together in vivo to control synaptic development (Zarnescu et al., 2005). Importantly, phosphorylation of dFmrp affects translational events (Ceman et al., 2003), suggesting that aPKC can regulate translation within the synapse, altering microtubule dynamics and hence synaptic architecture.

Do PAR-3 and PAR-6 also function with Fragile X protein? At the *Drosophila* NMJ, a fraction of PAR proteins have been observed to cosediment with an Lgl/dFmrp complex (Zarnescu et al., 2005). Baz (*Drosophila* PAR-3) and PAR-6 mutations enhanced Fragile X gain-of-function mutations, suggesting that Baz and PAR-6 may act to antagonize dFmrp function (Zarnescu et al., 2005). Whether Baz and PAR-6 have an effect on the developing synapse by regulating dFmrp remains to be tested.

aPKC in Synapse Development

As well as regulating Fmrp, aPKC may have other targets in synaptic development and function. For example, an autonomously active aPKC isoform, PKM, regulates long-term potentiation (LTP) in mice and persistent memory in *Drosophila* (Sessoms et al., 1992; Drier et al., 2002). Evidence from the developing *Drosophila* NMJ suggests that aPKC directly regulates microtubule dynamics presynaptically and both the actin and microtubule cytoskeletons postsynaptically (Ruiz-Canada et al., 2004). *Drosophila* aPKC protein is enriched both pre- and postsynaptically, particularly at microtubule-rich regions. A reduction in the number of newly formed synaptic boutons is evident in the neuromuscular junctions of flies carrying either hypomorphic alleles of aPKC or overexpressing PKM (Ruiz-Canada et al., 2004). Since both hypomorphic and constitutively active forms of aPKC cause a reduction in bouton number, normal synapse formation likely requires a very precise regulation of aPKC expression and activity.

Reduced aPKC activity causes microtubule fragmentation in the presynaptic bouton (Ruiz-Canada et al., 2004), similar to defects observed in Futsch mutant NMJs (Hummel et al., 2000; Roos et al., 2000). Conversely, overexpression of constitutively active aPKC resulted in longer microtubules and increased Futsch binding to microtubules in the presynaptic region, implicating aPKC in Futsch-mediated regulation of synaptic architecture (Ruiz-Canada et al., 2004). This is supported by biochemical and genetic experiments showing that Futsch recruits aPKC to tubulin (Ruiz-Canada et al., 2004). Together these data suggest that, presynaptically, aPKC interacts with Futsch to regulate microtubule stability.

Postsynaptically, aPKC has been shown to affect the dynamics of the actin cytoskeleton, leading to changes in GluRII localization, consistent with changes in synaptic efficacy (Ruiz-Canada et al., 2004). Hypomorphic aPKC mutants caused a reduction in actin localization but an increase in microtubule localization to the peribouton. By contrast, expression of constitutively active PKC postsynaptically led to an opposite phenotype: an increase in actin and a decrease in microtubules within the peribouton space (Ruiz-Canada et al., 2004).

Do aPKC-interacting proteins, in addition to dFmrp, Lgl, and Futsch, also play a synaptic role? The binding of PAR-3 and PAR-6 can, respectively, suppress or enhance aPKC activity (Lin et al., 2000; Yamanaka et al., 2001). Both PAR-3 and PAR-6 are enriched in the synaptic fraction of mammalian brain extracts (Lin et al., 2000). In addition, Baz, PAR-6, and aPKC coprecipitate from *Drosophila* NMJs, suggesting that these three proteins can form a complex at synapses (Ruiz-Canada et al., 2004). Immunocytochemical localization reveals that both Baz and PAR-6 are highly expressed presynaptically in the *Drosophila* NMJ, with PAR-6 partially colocalizing with aPKC along microtubules (Ruiz-Canada et al., 2004). Postsynaptically, Baz and PAR-6 are found within the peribouton region, a region devoid of aPKC (Ruiz-Canada et al., 2004).

Furthermore, reduced levels of functional aPKC cause a significant loss of both Baz and PAR-6 expression within the NMJ. A loss of actin localization at the postsynaptic peribouton region is observed in Baz mutants as well as aPKC hypomorphs, and alterations in Baz or PAR-6 levels lead to a loss of synaptic boutons (Ruiz-Canada et al., 2004). Together these data implicate aPKC in the development of the NMJ and suggest that Baz and PAR-6 also play a significant role in defining the architecture of the synapse, potentially by regulating aPKC activity and hence the actin and microtubule cytoskeletons. Further work will be required to elucidate the molecular details and to determine if these proteins have similar roles in mammalian synapse development.

Scribble and the Synapse

Observations from *Drosophila* epithelial cells have shown that Lgl functions genetically with two other tumor suppressor proteins, the MAGUK protein Discs large (Dlg) and the LAP protein Scribble (Bilder, 2004), which can interact through a third protein GUKholder (Mathew et al., 2002). Mutations in *Drosophila* Dlg lead to pronounced defects in the size of the synaptic bouton, the number of active zones, and changes in the postsynaptic membrane of the NMJ (Lahey et al., 1994; Budnik et al., 1996). In Dlg mutants, there is a loss of Scribble localization to the synaptic region (Mathew et al., 2002), whereas in Scribble mutants, Dlg localization is normal (Roche et al., 2002). Scribble mutants also show a less severe defect in synaptic morphology than Dlg mutants (Roche et al., 2002), suggesting that Dlg acts upstream of Scribble in synaptogenesis. A more extensive discussion of the role of Dlg in synapse development can be found in other reviews (Fujita and Kurachi, 2000; Montgomery et al., 2004).

The Scribble protein has N-terminal leucine-rich repeats followed by four PDZ domains, through which it binds a number of proteins including β-Pix, a GEF

for Cdc42, and the G protein-coupled receptor kinase-interacting protein 1 (Git1), an Arf GTPase-activating protein (ArfGAP) (Audebert et al., 2004).

Git1, Scribble, and Pix localize pre- and postsynaptically in mammalian neurons (Kim et al., 2003; Zhang et al., 2003; Audebert et al., 2004). In PC12 cells, dominant-negative mutations in either Scribble or Pix inhibit Ca^{2+}-dependent exocytosis, suggesting a role for this complex in neuronal transmission (Audebert et al., 2004). This phenotype is recapitulated by loss-of-function mutations in *Drosophila* Scribble, which lead to a redistribution of synaptic vesicles away from the active zone (Roche et al., 2002). Functionally, these synapses show defects in several forms of plasticity, including loss of facilitation and posttetanic potentiation, and also have defects in vesicle recycling (Roche et al., 2002). Git1 interacts with Pix, liprin-α, FAK, GRIP1, and Piccolo, raising the possibility that these proteins may form a complex to regulate the organization of the cytoskeleton matrix assembled at active zones (CAZ) (Kim et al., 2003). It will be interesting to determine whether Scribble is a part of this larger complex and can modulate the organization of the CAZ.

In addition to its presynaptic role, there is evidence that Scribble regulates postsynaptic architecture. *Drosophila* Scribble mutants have a decreased bouton size and a thickening of the basal lamina at the NMJ, suggesting that there are defects in the regulation of the actin cytoskeleton (Roche et al., 2002). In addition, recruitment of β-Pix to synapses along with Git1 is necessary for the formation and stabilization of synaptic spines, which are actin-rich processes (Zhang et al., 2003). A mutation in α-Pix, which prevents its interaction with Git1, has been linked with human mental retardation (Kutsche et al., 2000). Affected individuals have severely malformed synapses, reminiscent of Git1/Pix dominant-negative mutants (Kutsche et al., 2000).

Combined, these data suggest an intricate relationship between Scribble and the Git1/Pix complex in regulating the actin cytoskeleton, potentially via Cdc42 and Rac. Consistent with this idea is the finding that activated Rac associates with the Scribble and Pix complex following KCl-induced depolarization of PC12 cells (Audebert et al., 2004). Evidence from both *Drosophila* and mammalian systems has therefore begun to elucidate roles for polarity proteins in the development and maintenance of the synapse.

Conclusions

The PARs and their interacting proteins, known to regulate polarity in such varied systems as the *C. elegans* zygote, *Drosophila* neuroblasts, and mammalian epithelial cells, direct aspects of neuronal polarity, including axon specification and synaptogenesis. One of the main downstream effects of the polarity protein complexes is to regulate the actin and microtubule cytoskeletons. When considering axon specification, emerging evidence suggests an interesting interplay between known polarity proteins and proteins implicated in neuronal growth. Both seem to be interdependent, and future work will need to address the distinct roles that these two classes of protein play in neuronal polarity (Jiang and Rao, 2005). Further, it will be interesting to determine the role these polarity proteins have in the development and maintenance of dendrites. It will also be interesting to examine the relationship between axon and synapse development, since the same machinery appears to govern both processes. The influence of external signals is likely to trigger the switch that induces synaptogenesis. It is therefore critical that such studies be carried out using neurons in their native environment.

An additional phenomenon that is yet to be examined in neuronal polarity is the role of myosin in controlling actin dynamics. This has been shown to be regulated by PAR proteins in the *C. elegans* zygote (Munro et al., 2004). It will also be of interest to assess whether the Crumbs/Pals/PATJ or Dlg/Scribble/Lgl complexes, both of which are critical for epithelial polarity, are required in axon specification and if they interact in the same way in this system. Dlg, Scribble, and Lgl do have roles in synaptogenesis, and mice lacking Lgl1 have defects in neuronal differentiation, resulting in neonatal lethality (Klezovitch et al., 2004).

Proteins identified as interacting with polarity regulators in other systems may also play a role in neurons. For example, PAR-3 directly binds nectin-1 and -3 at the adherens junction in mouse neuroepithelial cells (Takekuni et al., 2003). Nectin-1 and -3 also have an important role in synapses, being localized to the presynaptic and postsynaptic membranes, respectively (Takai et al., 2003). These nectins, along with N-cadherin, are involved in the formation of puncta adherens junctions. It is therefore possible that PAR-3, by binding to the nectins, is also playing a role in this process.

It is becoming clear that polarity, in a wide range of different cell types, is regulated by a conserved set of proteins. Investigating the details of this system will give us a more complete understanding of a diverse range of processes including development, disease, and repair.

References

Audebert, S., Navarro, C., Nourry, C., Chasserot-Golaz, S., Lecine, P., Bellaiche, Y., Dupont, J.L., Premont, R.T., Sempere, C., Strub, J.M., et al. (2004). Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Curr. Biol. *14*, 987–995.

Benton, R., and St Johnston, D. (2003). A conserved oligomerization domain in *Drosophila* Bazooka/PAR-3 is important for apical localization and epithelial polarity. Curr. Biol. *13*, 1330–1334.

Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature *422*, 326–330.

Biernat, J., Wu, Y.Z., Timm, T., Zheng-Fischhofer, Q., Mandelkow, E., Meijer, L., and Mandelkow, E.M. (2002). Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity. Mol. Biol. Cell *13*, 4013–4028.

Bienz, M. (1999). APC: the plot thickens. Curr. Opin. Genet. Dev. *9*, 595–603.

Bienz, M. (2002). The subcellular destinations of APC proteins. Nat. Rev. Mol. Cell Biol. *3*, 328–338.

Bilder, D. (2004). Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. Genes Dev. *18*, 1909–1925.

Bilder, D., Schober, M., and Perrimon, N. (2003). Integrated activity

of PDZ protein complexes regulates epithelial polarity. Nat. Cell Biol. *5*, 53–58.

Bradke, F., and Dotti, C.G. (1999). The role of local actin instability in axon formation. Science *283*, 1931–1934.

Budnik, V., Koh, Y.H., Guan, B., Hartmann, B., Hough, C., Woods, D., and Gorczyca, M. (1996). Regulation of synapse structure and function by the *Drosophila* tumor suppressor gene *dlg*. Neuron *17*, 627–640.

Ceman, S., O'Donnell, W.T., Reed, M., Patton, S., Pohl, J., and Warren, S.T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. Hum. Mol. Genet. *12*, 3295–3305.

Chen, X., and Macara, I.G. (2005). PAR-3 controls tight junction assembly through the Rac exchange factor Tiam1. Nat. Cell Biol. *7*, 262–269.

Chiurazzi, P., Neri, G., and Oostra, B.A. (2003). Understanding the biological underpinnings of fragile X syndrome. Curr. Opin. Pediatr. *15*, 559–566.

Chou, M.M., Hou, W., Johnson, J., Graham, L.K., Lee, M.H., Chen, C.S., Newton, A.C., Schaffhausen, B.S., and Toker, A. (1998). Regulation of protein kinase C zeta by PI 3-kinase and PDK-1. Curr. Biol. *8*, 1069–1077.

Clements, W.K., and Kimelman, D. (2003). Wnt signalling gets XEEKy. Nat. Cell Biol. *5*, 861–863.

Cole, A.R., Knebel, A., Morrice, N.A., Robertson, L.A., Irving, A.J., Connolly, C.N., and Sutherland, C. (2004). GSK-3 phosphorylation of the Alzheimer epitope within collapsin response mediator proteins regulates axon elongation in primary neurons. J. Biol. Chem. *279*, 50176–50180.

Cowan, C.R., and Hyman, A.A. (2004). Asymmetric cell division in *C. elegans*: cortical polarity and spindle positioning. Annu. Rev. Cell Dev. Biol. *20*, 427–453.

Crump, J.G., Zhen, M., Jin, Y., and Bargmann, C.I. (2001). The SAD-1 kinase regulates presynaptic vesicle clustering and axon termination. Neuron *29*, 115–129.

Davies, S.P., Reddy, H., Caivano, M., and Cohen, P. (2000). Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem. J. *351*, 95–105.

Dotti, C.G., Sullivan, C.A., and Banker, G.A. (1988). The establishment of polarity by hippocampal neurons in culture. J. Neurosci. *8*, 1454–1468.

Dresbach, T., Qualmann, B., Kessels, M.M., Garner, C.C., and Gundelfinger, E.D. (2001). The presynaptic cytomatrix of brain synapses. Cell. Mol. Life Sci. *58*, 94–116.

Drier, E.A., Tello, M.K., Cowan, M., Wu, P., Blace, N., Sacktor, T.C., and Yin, J.C. (2002). Memory enhancement and formation by atypical PKM activity in *Drosophila melanogaster*. Nat. Neurosci. *5*, 316–324.

Edwards, D.C., Sanders, L.C., Bokoch, G.M., and Gill, G.N. (1999). Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat. Cell Biol. *1*, 253–259.

Eickholt, B.J., Walsh, F.S., and Doherty, P. (2002). An inactive pool of GSK-3 at the leading edge of growth cones is implicated in Semaphorin 3A signaling. J. Cell Biol. *157*, 211–217.

Etemad-Moghadam, B., Guo, S., and Kemphues, K.J. (1995). Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early *C. elegans* embryos. Cell *83*, 743–752.

Etienne-Manneville, S., and Hall, A. (2003). Cdc42 regulates GSK-3β and adenomatous polyposis coli to control cell polarity. Nature *421*, 753–756.

Fan, S., Hurd, T.W., Liu, C.J., Straight, S.W., Weimbs, T., Hurd, E.A., Domino, S.E., and Margolis, B. (2004). Polarity proteins control ciliogenesis via kinesin motor interactions. Curr. Biol. *14*, 1451–1461.

Fortini, M.E. (2004). PAR-1 for the course of neurodegeneration. Cell *116*, 631–632.

Frame, S., and Cohen, P. (2001). GSK3 takes centre stage more than 20 years after its discovery. Biochem. J. *359*, 1–16.

Franco, B., Bogdanik, L., Bobinnec, Y., Debec, A., Bockaert, J., Parmentier, M.L., and Grau, Y. (2004). Shaggy, the homolog of glycogen synthase kinase *3*, controls neuromuscular junction growth in *Drosophila*. J. Neurosci. *24*, 6573–6577.

Fujita, A., and Kurachi, Y. (2000). SAP family proteins. Biochem. Biophys. Res. Commun. *269*, 1–6.

Fukata, Y., Kimura, T., and Kaibuchi, K. (2002a). Axon specification in hippocampal neurons. Neurosci. Res. *43*, 305–315.

Fukata, Y., Itoh, T.J., Kimura, T., Menager, C., Nishimura, T., Shiromizu, T., Watanabe, H., Inagaki, N., Iwamatsu, A., Hotani, H., and Kaibuchi, K. (2002b). CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat. Cell Biol. *4*, 583–591.

Garrard, S.M., Capaldo, C.T., Gao, L., Rosen, M.K., Macara, I.G., and Tomchick, D.R. (2003). Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein, Par6. EMBO J. *22*, 1125–1133.

Goold, R.G., Owen, R., and Gordon-Weeks, P.R. (1999). Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. J. Cell Sci. *112*, 3373–3384.

Govek, E.E., Newey, S.E., and Van Aelst, L. (2005). The role of the Rho GTPases in neuronal development. Genes Dev. *19*, 1–49.

Guo, S., and Kemphues, K.J. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell *81*, 611–620.

Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R.D., Krishna, U.M., Falck, J.R., White, M.A., and Broek, D. (1998). Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. Science *279*, 558–560.

Harris, T.J., and Peifer, M. (2004). Adherens junction-dependent and -independent steps in the establishment of epithelial cell polarity in *Drosophila*. J. Cell Biol. *167*, 135–147.

Harwood, A., and Braga, V.M. (2003). Cdc42 & GSK-3: signals at the crossroads. Nat. Cell Biol. *5*, 275–277.

Hers, I., Tavare, J.M., and Denton, R.M. (1999). The protein kinase C inhibitors bisindolylmaleimide I (GF 109203x) and IX (Ro 31–8220) are potent inhibitors of glycogen synthase kinase-3 activity. FEBS Lett. *460*, 433–436.

Horton, A.C., and Ehlers, M.D. (2003). Neuronal polarity and trafficking. Neuron *40*, 277–295.

Hummel, T., Krukkert, K., Roos, J., Davis, G., and Klambt, C. (2000). *Drosophila* Futsch/22C10 is a MAP1B-like protein required for dendritic and axonal development. Neuron *26*, 357–370.

Hung, T.J., and Kemphues, K.J. (1999). PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos. Development *126*, 127–135.

Hurd, T.W., and Margolis, B. (2005). Pars and polarity: taking control of Rac. Nat. Cell Biol. *7*, 205–207.

Hurd, T.W., Gao, L., Roh, M.H., Macara, I.G., and Margolis, B. (2003a). Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. Nat. Cell Biol. *5*, 137–142.

Hurd, T.W., Fan, S., Liu, C.J., Kweon, H.K., Hakansson, K., and Margolis, B. (2003b). Phosphorylation-dependent binding of 14–3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia. Curr. Biol. *13*, 2082–2090.

Hurov, J.B., Watkins, J.L., and Piwnica-Worms, H. (2004). Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity. Curr. Biol. *14*, 736–741.

Imamura, T., Huang, J., Usui, I., Satoh, H., Bever, J., and Olefsky, J.M. (2003). Insulin-induced GLUT4 translocation involves protein kinase C-lambda-mediated functional coupling between Rab4 and the motor protein kinesin. Mol. Cell. Biol. *23*, 4892–4900.

Inagaki, N., Chihara, K., Arimura, N., Menager, C., Kawano, Y., Matsuo, N., Nishimura, T., Amano, M., and Kaibuchi, K. (2001). CRMP-2 induces axons in cultured hippocampal neurons. Nat. Neurosci. *4*, 781–782.

Irazoqui, J.E., Gladfelter, A.S., and Lew, D.J. (2003). Scaffold-mediated symmetry breaking by Cdc42p. Nat. Cell Biol. *5*, 1062–1070.

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K.J., and Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. J. Cell Biol. *143*, 95–106.

Jiang, H., and Rao, Y. (2005). Axon formation: fate versus growth. Nat. Neurosci. *8*, 544–546.

Jiang, H., Guo, W., Liang, X., and Rao, Y. (2005). Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3β and its upstream regulators. Cell *120*, 123–135.

Joberty, G., Petersen, C., Gao, L., and Macara, I.G. (2000). The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat. Cell Biol. *2*, 531–539.

Johansson, A., Driessens, M., and Aspenstrom, P. (2000). The mammalian homologue of the *Caenorhabditis elegans* polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac1. J. Cell Sci. *113*, 3267–3275.

Jope, R.S., and Johnson, G.V. (2004). The glamour and gloom of glycogen synthase kinase-3. Trends Biochem. Sci. *29*, 95–102.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nat. Rev. Neurosci. *5*, 771–781.

Kim, S., Ko, J., Shin, H., Lee, J.R., Lim, C., Han, J.H., Altrock, W.D., Garner, C.C., Gundelfinger, E.D., Premont, R.T., et al. (2003). The GIT family of proteins forms multimers and associates with the presynaptic cytomatrix protein Piccolo. J. Biol. Chem. *278*, 6291–6300.

Kishi, M., Pan, Y.A., Crump, J.G., and Sanes, J.R. (2005). Mammalian SAD kinases are required for neuronal polarization. Science *307*, 929–932.

Klezovitch, O., Fernandez, T.E., Tapscott, S.J., and Vasioukhin, V. (2004). Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice. Genes Dev. *18*, 559–571.

Kooy, R.F. (2003). Of mice and the fragile X syndrome. Trends Genet. *19*, 148–154.

Kunda, P., Paglini, G., Quiroga, S., Kosik, K., and Caceres, A. (2001). Evidence for the involvement of Tiam1 in axon formation. J. Neurosci. *21*, 2361–2372.

Kusakabe, M., and Nishida, E. (2004). The polarity-inducing kinase PAR-1 controls *Xenopus* gastrulation in cooperation with 14–3-3 and aPKC. EMBO J. *23*, 4190–4201.

Kutsche, K., Yntema, H., Brandt, A., Jantke, I., Nothwang, H.G., Orth, U., Boavida, M.G., David, D., Chelly, J., Fryns, J.P., et al. (2000). Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. Nat. Genet. *26*, 247–250.

Lahey, T., Gorczyca, M., Jia, X.X., and Budnik, V. (1994). The *Drosophila* tumor suppressor gene *dlg* is required for normal synaptic bouton structure. Neuron *13*, 823–835.

Le Good, J.A., Ziegler, W.H., Parekh, D.B., Alessi, D.R., Cohen, P., and Parker, P.J. (1998). Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science *281*, 2042–2045.

Levitan, D.J., Boyd, L., Mello, C.C., Kemphues, K.J., and Stinchcomb, D.T. (1994). *par-2*, a gene required for blastomere asymmetry in *Caenorhabditis elegans*, encodes zinc-finger and ATP-binding motifs. Proc. Natl. Acad. Sci. USA *91*, 6108–6112.

Lin, D., Edwards, A.S., Fawcett, J.P., Mbamalu, G., Scott, J.D., and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat. Cell Biol. *2*, 540–547.

Lin-Marq, N., Borel, C., and Antonarakis, S.E. (2005). Peutz-Jeghers LKB1 mutants fail to activate GSK-3β, preventing it from inhibiting Wnt signaling. Mol. Genet. Genomics *273*, 184–196.

Lizcano, J.M., Goransson, O., Toth, R., Deak, M., Morrice, N.A., Boudeau, J., Hawley, S.A., Udd, L., Makela, T.P., Hardie, D.G., and Alessi, D.R. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J. *23*, 833–843.

Luo, L., Liao, Y.J., Jan, L.Y., and Jan, Y.N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. Genes Dev. *8*, 1787–1802.

Macara, I.G. (2004). Par proteins: partners in polarization. Curr. Biol. *14*, R160–R162.

Mandell, J.W., and Banker, G.A. (1996a). Microtubule-associated proteins, phosphorylation gradients, and the establishment of neuronal polarity. Perspect. Dev. Neurobiol. *4*, 125–135.

Mandell, J.W., and Banker, G.A. (1996b). A spatial gradient of tau protein phosphorylation in nascent axons. J. Neurosci. *16*, 5727–5740.

Martin, K.C. (2004). Local protein synthesis during axon guidance and synaptic plasticity. Curr. Opin. Neurobiol. *14*, 305–310.

Mathew, D., Gramates, L.S., Packard, M., Thomas, U., Bilder, D., Perrimon, N., Gorczyca, M., and Budnik, V. (2002). Recruitment of Scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein. Curr. Biol. *12*, 531–539.

Matsumine, A., Ogai, A., Senda, T., Okumura, N., Satoh, K., Baeg, G.H., Kawahara, T., Kobayashi, S., Okada, M., Toyoshima, K., and Akiyama, T. (1996). Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. Science *272*, 1020–1023.

Menager, C., Arimura, N., Fukata, Y., and Kaibuchi, K. (2004). PIP3 is involved in neuronal polarization and axon formation. J. Neurochem. *89*, 109–118.

Montgomery, J.M., Zamorano, P.L., and Garner, C.C. (2004). MAGUKs in synapse assembly and function: an emerging view. Cell. Mol. Life Sci. *61*, 911–929.

Morton, D.G., Shakes, D.C., Nugent, S., Dichoso, D., Wang, W., Golden, A., and Kemphues, K.J. (2002). The *Caenorhabditis elegans par-5* gene encodes a 14–3-3 protein required for cellular asymmetry in the early embryo. Dev. Biol. *241*, 47–58.

Munro, E., Nance, J., and Priess, J.R. (2004). Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo. Dev. Cell *7*, 413–424.

Nam, S.C., and Choi, K.W. (2003). Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. Development *130*, 4363–4372.

Nishimura, T., Fukata, Y., Kato, K., Yamaguchi, T., Matsuura, Y., Kamiguchi, H., and Kaibuchi, K. (2003). CRMP-2 regulates polarized Numb-mediated endocytosis for axon growth. Nat. Cell Biol. *5*, 819–826.

Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S., and Kaibuchi, K. (2004a). Role of the PAR-3–KIF3 complex in the establishment of neuronal polarity. Nat. Cell Biol. *6*, 328–334.

Nishimura, I., Yang, Y., and Lu, B. (2004b). PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in *Drosophila*. Cell *116*, 671–682.

Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y., Ohno, S., Hoshino, M., and Kaibuchi, K. (2005). PAR-6-PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. Nat. Cell Biol. *7*, 270–277.

Oriente, F., Formisano, P., Miele, C., Fiory, F., Maitan, M.A., Vigliotta, G., Trencia, A., Santopietro, S., Caruso, M., Van Obberghen, E., and Beguinot, F. (2001). Insulin receptor substrate-2 phosphorylation is necessary for protein kinase C ζ activation by insulin in L6hIR cells. J. Biol. Chem. *276*, 37109–37119.

Ossipova, O., Bardeesy, N., DePinho, R.A., and Green, J.B. (2003). LKB1 (XEEK1) regulates Wnt signalling in vertebrate development. Nat. Cell Biol. *5*, 889–894.

Patel, S., Doble, B., and Woodgett, J.R. (2004). Glycogen synthase kinase-3 in insulin and Wnt signalling: a double-edged sword? Biochem. Soc. Trans. *32*, 803–808.

Plant, P.J., Fawcett, J.P., Lin, D.C., Holdorf, A.D., Binns, K., Kulkarni, S., and Pawson, T. (2003). A polarity complex of mPAR-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. Nat. Cell Biol. *5*, 301–308.

Qiu, R.G., Abo, A., and Steven Martin, G. (2000). A human homolog

of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCζ signaling and cell transformation. Curr. Biol. *10*, 697–707.

Ramakers, G.J. (2002). Rho proteins, mental retardation and the cellular basis of cognition. Trends Neurosci. *25*, 191–199.

Rao, A., and Craig, A.M. (2000). Signaling between the actin cytoskeleton and the postsynaptic density of dendritic spines. Hippocampus *10*, 527–541.

Roche, J.P., Packard, M.C., Moeckel-Cole, S., and Budnik, V. (2002). Regulation of synaptic plasticity and synaptic vesicle dynamics by the PDZ protein Scribble. J. Neurosci. *22*, 6471–6479.

Rolls, M.M., and Doe, C.Q. (2004). Baz, PAR-6 and aPKC are not required for axon or dendrite specification in *Drosophila*. Nat. Neurosci. *7*, 1293–1295.

Roos, J., Hummel, T., Ng, N., Klambt, C., and Davis, G.W. (2000). *Drosophila* Futsch regulates synaptic microtubule organization and is necessary for synaptic growth. Neuron *26*, 371–382.

Rosso, S., Bollati, F., Bisbal, M., Peretti, D., Sumi, T., Nakamura, T., Quiroga, S., Ferreira, A., and Caceres, A. (2004). LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons. Mol. Biol. Cell *15*, 3433–3449.

Ruiz-Canada, C., Ashley, J., Moeckel-Cole, S., Drier, E., Yin, J., and Budnik, V. (2004). New synaptic bouton formation is disrupted by misregulation of microtubule stability in aPKC mutants. Neuron *42*, 567–580.

Santolini, E., Puri, C., Salcini, A.E., Gagliani, M.C., Pelicci, P.G., Tacchetti, C., and Di Fiore, P.P. (2000). Numb is an endocytic protein. J. Cell Biol. *151*, 1345–1352.

Santos Da Silva, J., Schubert, V., and Dotti, C.G. (2004). RhoA, Rac1, and cdc42 intracellular distribution shift during hippocampal neuron development. Mol. Cell. Neurosci. *27*, 1–7.

Santos Da Silva, J., Hasegawa, T., Miyagi, T., Dotti, C.G., and Abad-Rodriguez, J. (2005). Asymmetric membrane ganglioside sialidase activity specifies axonal fate. Nat. Neurosci. *8*, 606–615.

Schaefer, A.M., and Nonet, M.L. (2001). Cellular and molecular insights into presynaptic assembly. Curr. Opin. Neurobiol. *11*, 127–134.

Schenck, A., Bardoni, B., Langmann, C., Harden, N., Mandel, J.L., and Giangrande, A. (2003). CYFIP/Sra-1 controls neuronal connectivity in *Drosophila* and links the Rac1 GTPase pathway to the fragile X protein. Neuron *38*, 887–898.

Schwamborn, J.C., and Puschel, A.W. (2004). The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. Nat. Neurosci. *7*, 923–929.

Sessoms, J.S., Chen, S.J., Chetkovich, D.M., Powell, C.M., Roberson, E.D., Sweatt, J.D., and Klann, E. (1992). Ca(2+)-induced persistent protein kinase C activation in rat hippocampal homogenates. Second Messengers Phosphoproteins *14*, 109–126.

Setou, M., Hayasaka, T., and Yao, I. (2004). Axonal transport versus dendritic transport. J. Neurobiol. *58*, 201–206.

Shi, S.H., Jan, L.Y., and Jan, Y.N. (2003). Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. Cell *112*, 63–75.

Shi, S.H., Cheng, T., Jan, L.Y., and Jan, Y.N. (2004). APC and GSK-3β are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. Curr. Biol. *14*, 2025–2032.

Shimomura, A., Kohu, K., Akiyama, T., and Senda, T. (2005). Subcellular localization of the tumor suppressor protein APC in developing cultured neurons. Neurosci. Lett. *375*, 81–86.

Solecki, D.J., Model, L., Gaetz, J., Kapoor, T.M., and Hatten, M.E. (2004). Par6α signaling controls glial-guided neuronal migration. Nat. Neurosci. *7*, 1195–1203.

Spicer, J., Rayter, S., Young, N., Elliott, R., Ashworth, A., and Smith, D. (2003). Regulation of the Wnt signalling component PAR1A by the Peutz-Jeghers syndrome kinase LKB1. Oncogene *22*, 4752–4756.

Stoothoff, W.H., and Johnson, G.V. (2005). Tau phosphorylation: physiological and pathological consequences. Biochim. Biophys. Acta *1739*, 280–297.

Sun, T.Q., Lu, B., Feng, J.J., Reinhard, C., Jan, Y.N., Fantl, W.J., and Williams, L.T. (2001). PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. Nat. Cell Biol. *3*, 628–636.

Suzuki, A., Hirata, M., Kamimura, K., Maniwa, R., Yamanaka, T., Mizuno, K., Kishikawa, M., Hirose, H., Amano, Y., Izumi, N., et al. (2004). aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity. Curr. Biol. *14*, 1425–1435.

Takai, Y., Shimizu, K., and Ohtsuka, T. (2003). The roles of cadherins and nectins in interneuronal synapse formation. Curr. Opin. Neurobiol. *13*, 520–526.

Takekuni, K., Ikeda, W., Fujito, T., Morimoto, K., Takeuchi, M., Monden, M., and Takai, Y. (2003). Direct binding of cell polarity protein PAR-3 to cell-cell adhesion molecule nectin at neuroepithelial cells of developing mouse. J. Biol. Chem. *278*, 5497–5500.

Tanentzapf, G., and Tepass, U. (2003). Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization. Nat. Cell Biol. *5*, 46–52.

Timm, T., Li, X.Y., Biernat, J., Jiao, J., Mandelkow, E., Vandekerckhove, J., and Mandelkow, E.M. (2003). MARKK, a Ste20-like kinase, activates the polarity-inducing kinase MARK/PAR-1. EMBO J. *22*, 5090–5101.

Trivedi, N., Marsh, P., Goold, R.G., Wood-Kaczmar, A., and Gordon-Weeks, P.R. (2005). Glycogen synthase kinase-3β phosphorylation of MAP1B at Ser1260 and Thr1265 is spatially restricted to growing axons. J. Cell Sci. *118*, 993–1005.

von Stein, W., Ramrath, A., Grimm, A., Muller-Borg, M., and Wodarz, A. (2005). Direct association of Bazooka/PAR-3 with the lipid phosphatase PTEN reveals a link between the PAR/aPKC complex and phosphoinositide signaling. Development *132*, 1675–1686.

Wang, F., Herzmark, P., Weiner, O.D., Srinivasan, S., Servant, G., and Bourne, H.R. (2002). Lipid products of PI(3)Ks maintain persistent cell polarity and directed motility in neutrophils. Nat. Cell Biol. *4*, 513–518.

Wang, Q., Hurd, T.W., and Margolis, B. (2004). Tight junction protein Par6 interacts with an evolutionarily conserved region in the amino terminus of PALS1/stardust. J. Biol. Chem. *279*, 30715–30721.

Watts, J.L., Morton, D.G., Bestman, J., and Kemphues, K.J. (2000). The *C. elegans par-4* gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. Development *127*, 1467–1475.

Weiner, O.D., Neilsen, P.O., Prestwich, G.D., Kirschner, M.W., Cantley, L.C., and Bourne, H.R. (2002). A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity. Nat. Cell Biol. *4*, 509–513.

Welch, H.C., Coadwell, W.J., Ellson, C.D., Ferguson, G.J., Andrews, S.R., Erdjument-Bromage, H., Tempst, P., Hawkins, P.T., and Stephens, L.R. (2002). P-Rex1, a PtdIns(3,4,5)P3- and Gbetagamma-regulated guanine-nucleotide exchange factor for Rac. Cell *108*, 809–821.

Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H., and Ohno, S. (2001). PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. Genes Cells *6*, 721–731.

Yamanaka, T., Horikoshi, Y., Sugiyama, Y., Ishiyama, C., Suzuki, A., Hirose, T., Iwamatsu, A., Shinohara, A., and Ohno, S. (2003). Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. Curr. Biol. *13*, 734–743.

Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A., and Kaibuchi, K. (2005). GSK-3β regulates phosphorylation of CRMP-2 and neuronal polarity. Cell *120*, 137–149.

Zarnescu, D.C., Jin, P., Betschinger, J., Nakamoto, M., Wang, Y., Dockendorff, T.C., Feng, Y., Jongens, T.A., Sisson, J.C., Knoblich, J.A., et al. (2005). Fragile X protein functions with lgl and the par complex in flies and mice. Dev. Cell *8*, 43–52.

Zhang, Y.Q., and Broadie, K. (2005). Fathoming fragile X in fruit flies. Trends Genet. *21*, 37–45.

Zhang, Y.Q., Bailey, A.M., Matthies, H.J., Renden, R.B., Smith, M.A., Speese, S.D., Rubin, G.M., and Broadie, K. (2001). *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell *107*, 591–603.

Zhang, H., Webb, D.J., Asmussen, H., and Horwitz, A.F. (2003). Synapse formation is regulated by the signaling adaptor GIT1. J. Cell Biol. *161*, 131–142.

Zhou, F.Q., Zhou, J., Dedhar, S., Wu, Y.H., and Snider, W.D. (2004). NGF-induced axon growth is mediated by localized inactivation of GSK-3β and functions of the microtubule plus end binding protein APC. Neuron *42*, 897–912.

Ziff, E.B. (1997). Enlightening the postsynaptic density. Neuron *19*, 1163–1174.

Ziv, N.E., and Garner, C.C. (2004). Cellular and molecular mechanisms of presynaptic assembly. Nat. Rev. Neurosci. *5*, 385–399.

Zumbrunn, J., Kinoshita, K., Hyman, A.A., and Nathke, I.S. (2001). Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3β phosphorylation. Curr. Biol. *11*, 44–49.